Pathophysiological basis of cerebral vasospasm following aneurysmal subarachnoid haemorrhage

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Subarachnoid haemorrhage (SAH) following rupture of an intracranial aneurysm is associated with a greater than 35% mortality within the first 3 months. Delayed ischaemia due to cerebral vasospasm (CVSP) occurs in approximately one-third of all patients with this disease and accounts for over 20% of its overall morbidity and mortality. Although the clinical and experimental features of CVSP have been widely documented, the pathophysiology remains controversial. Its basis, however, is almost certainly multifactorial. The cerebrovascular microenvironment is significantly altered as a consequence of haemorrhage into the subarachnoid space. Important putative factors implicated in this change include oxyhaemoglobin, endothelin, oxygen- derived free radicals, and neurovascular nitric oxide, arachidonic acid and carbon monoxide systems. In addition, distinct histological changes related to the overall physiological response occur in cerebral vessels undergoing spasm. The present work aims to detail and unify our current understanding of the biological mechanisms underlying this devastating condition.

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Introduction

Cerebrovascular spasm is a prominent delayed pathological response to aneurysmal subarachnoid haemorrhage (SAH).[1] Although it is probable that in almost all SAH patients there is a certain degree of arterial narrowing, this may or may not be symptomatic.[2,3] In a recent analysis of more than 1000 reports of this condition, the incidence of symptomatic vasospasm was found to be 32.5%, the mean time of onset being 7.7 days after the ictus.[3] Overall, its occurrence was reported to be associated with a mortality of 30%, with permanent neurological deficits due to cerebral infarction occurring in almost half of the remaining patients.[3] It should be noted that the severity of delayed ischaemia due to cerebral vasospasm (CVSP) may be greater among patients admitted in poorer neurological grades.[4]

Despite extensive clinical and experimental investigation, the biological mechanisms underlying posthaemorrhagic arterial narrowing remain poorly understood.[5,6] A wide variety of factors have been implicated as putative mediators of this response, however their individual roles and interactions remain both complex and controversial.[7] In order to simplify a description of the pathophysiology of CVSP, the cerebrovascular microenvironment may be divided into four primary compartments (Fig. 1). Each compartment plays a fundamental role in modulating the resting tone of the cerebral blood vessel and undergoes significant physiological and structural alteration following SAH. Important putative mediators of arterial tone according to their physiological compartment of origin are listed in Table 1.

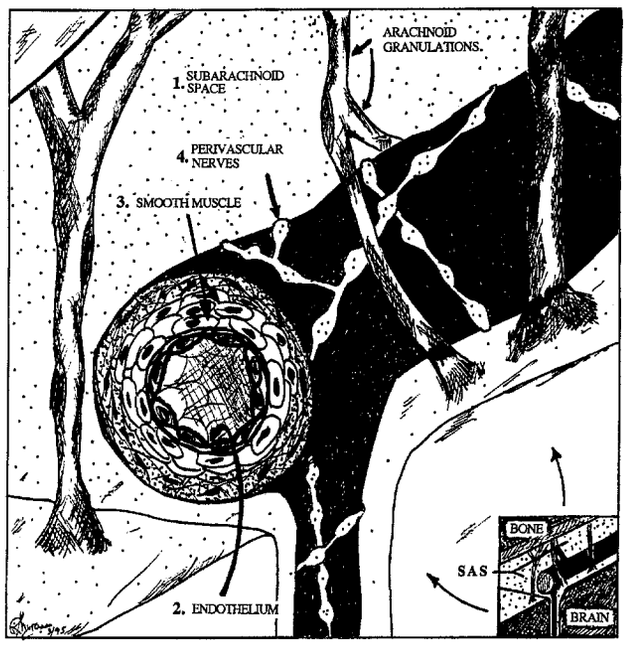

Fig. 1 The cerebrovascular microenvironment. Its four primary compartments are: (1) subarachnoid space; (2) vascular endothelium; (3) vascular smooth muscle; and (4) adventitial nerves. Each compartment is physiologically and structurally altered following aneurysmal SAH. The inset shows a cerebral blood vessel coursing through the subarachnoid space.

Table 1 Mediators of arterial tone by physiological compartment

Subarachnoid space	Vascular smooth muscle	Vascular endothelium	Adventitial nerves
Oxyhaemoglobin	Contractile apparatus	Nitric oxide	Adrenergic innervation
Aggregating platelets	Inducible nitric oxide synthase	Endothelin-1	NANC nerve fibres
Circulating Ca^{2+} and Mg^{2+}	Calcium and potassium channels	Oxygen derived free radicals	Carbon monoxide
		Arachidonic acid	Calcitonin gene related peptide

Given the significant morbidity and mortality associated with CVSP, substantial effort continues to be directed towards elucidating its pathophysiology. The present work aims to detail and unify our current understanding of the mechanisms underlying this life threatening condition, and is divided into three parts. In part 1, important posthaemorrhagic physiological changes in the cerebrovascular microenvironment are detailed, while part 2 describes their structural accompaniments. The final part presents a hypothesis binding these physiological and histopathological sequelae. For a review of the clinical aspects of CVSP the reader is referred to the recent articles by Dorsch.[8–10]

Part 1 – Altered physiological compartments

It is widely accepted that the release of intracellular products from the lysis of subarachnoid blood clot provides the initial trigger for arterial constriction.[1] These products and the subcellular systems they modulate are detailed below.

Subarachnoid space

Oxyhaemoglobin

The release of oxyhaemoglobin after erythrocyte breakdown plays a critical role in the induction of posthaemorrhagic arterial narrowing.[1, 11–14] Intracisternal injection of blood lacking erythrocytes does not produce angiographic or structural arterial narrowing,[7] while exposing cerebral vessels to extracts of lysed red blood cells or oxyhaemoglobin causes sustained contraction.[1,7] In terms of its actions on blood vessels, haemoglobin causes direct endothelial cell damage, myonecrosis and transformation of adventitial nerve endings, effects which have been found to be time- and dose-dependent.[14] The cytotoxicity of haemoglobin may be related to its ability to enhance lipid peroxidation, liberate arachidonic acid (AA), and form oxygen derived free radicals, including superoxide.[1,14] In support of these findings Takenaka et al.,[14] using cultured bovine cerebrovascular endothelium, have shown that the cellular damage induced by exposure to haemoglobin could be significantly reduced in the presence of superoxide dismutase (SOD), a scavenger of superoxide, or 4-Bromophenacyl-bromide, an inhibitor of AA biosynthesis. As will be elaborated further, other vasculopathic actions of haemoglobin include its augmentation of cytokine production, including interleukin-1β (IL-1β),[11] its ability to scavenge endothelium derived relaxing factor (EDRF) while facilitating the production of both endothelin and vasoconstrictive prostaglandins,[1,13] and its potentiation of noradrenaline- and serotonin-induced vasoconstriction.[1,12]

Aggregating platelets

Under normal conditions the endothelium releases substances such as EDRF (see below) and prostacyclin (PGI_2), both of which relax vascular smooth muscle and inhibit the aggregation of platelets.[15] Following SAH, however, impairment of PGI_2 and EDRF action leads to disinhibition of platelet aggregation.[16,17] Under these conditions substances released by the platelet aggregate, including adenosine di- and tri-phosphate, serotonin and thrombin, cause vascular smooth muscle contraction.[15,18] In addition, the release of platelet derived growth factor (PDGF) stimulates proliferation of cells within the tunica media.[7] Clinically, a recent study found that in a certain subgroup of aneurysmal SAH patients, the use of the anti-platelet agent, aspirin, prior to the ictus protected against the occurrence of delayed ischaemic deficit, although it also increased the risk of rebleeding.[19]

Circulating calcium and magnesium

Endothelium-dependent vascular smooth muscle relaxation is a function of both the presence of extracellular calcium and the intra*endothelial* calcium concentration.[20] It is likely that the influx of calcium into endothelial cells is coupled to the synthesis and release of EDRF (see below).[21,22] By contrast, extracellular magnesium has been shown to inhibit the influx of calcium into vascular myocytes,[23] myocardial cells,[24] and hippocampal neurons.[25] It may also interfere with calcium release from stores within vascular smooth muscle.[26] In experiments using precontracted bovine pulmonary vessels, Gold et al[20] report that rapid removal of magnesium from the extracellular solution causes a transient endothelium- and calcium-dependent relaxation that is associated with elevated levels of cyclic 3'5'-guanosine monophosphate (cGMP). Oxyhaemoglobin and superoxide were found to have antagonized this response, while vasodilation was enhanced in the presence of SOD. The transient relaxation was followed by sustained endothelium-*in*dependent contraction, an effect attributed to the unopposed smooth muscle actions of excess intracellular calcium.[20]

Vascular endothelium

Endothelium derived nitric oxide

In 1980 Furchgott and Zawadzki[27] demonstrated that the vascular relaxation induced by acetylcholine was dependent

Table 2 Properties of endothelium derived nitric oxide

Biosynthesis	L-arginine via calcium-dependent NOS
Principal sites of synthesis	Vascular endothelium; perivascular nerves
Release	Pulsatile flow, shear stress; or receptor-mediated
Mechanism of action	Stimulates guanylate cyclase to raise cGMP level
Water solubility	Limited
Transmembrane diffusibility	Very high; comparable to O_2 and CO_2
Half-life	2–4 seconds
Stability	Highly reactive and unstable free radical species
Oxidation	Rapid; converted to nitrite and nitrate
Endogenous source	L-arginine
Exogenous sources	Sodium nitroprusside; glyceryl trinitrate
Biosynthesis inhibitors	L-NMMA; L-NAME (see text)
Inactivators	Haemoglobin; oxygen derived free radicals
Body distribution	Widespread; particularly CNS, CVS and GIT
Proposed cardiovascular role	Vascular smooth muscle relaxation
Other functions	Hippocampal long-term potentiation; GIT motility
Closest structural and functional relation	CO

CNS = central nervous system, CVS = cardiovascular system, CO = carbon monoxide, GIT = gastrointestinal tract, NOS = nitric oxide synthase.

upon the presence of a factor derived from the endothelial lining of blood vessels, and so named it EDRF. By 1987, EDRF and nitric oxide (NO) were known to be the same molecule.[28,29] The characteristics of NO are given in Table 2. Importantly, three pivotal vasoregulatory functions of NO include smooth muscle relaxation,[30] inhibition of platelet aggregation,[16] and prevention of smooth muscle cell proliferation.[31,32] NO mediates vasodilation through its ability to decrease the level of intrasarcoplasmic calcium via a yet uncharacterized pathway involving increased levels of cGMP.[21] A recent study trialing the effect of intracarotid infusion of NO in primate vasospastic arteries has shown that such a procedure is associated with an increase in cerebral blood flow, significant reversal of angiographic spasm and minimal systemic hypotension.[33]

The presence of constitutive nitric oxide synthase (cNOS) and the synthesis of NO have now been demonstrated in a wide variety of systems throughout the body.[21,34] Important characteristics of cNOS include its presence in vascular endothelium and perivascular neurons,[21,34,35] its calcium dependence[22] and its structural similarity to the enzyme cytochrome p-450 reductase.[36,37] NOS has recognition sites for calmodulin, NADPH and cAMP-dependent protein kinase C (PKC).[36] Further, the activity of NOS is inhibited by L-N^g-monomethylarginine (L-NMMA) and L-N^g-nitroarginine methylester (L-NAME).[38–42] Although haemoglobin does not directly modulate NOS, it *does* inactivate both NO and guanylate cyclase (GCase).[(21)] Finally, it should be noted that the cerebrovascular L-arginine:NO pathway mediates the vasoregulatory response to agents such as vasopressin and bradykinin.[32,43–46]

Endothelin-1

Of the three endothelin (ET) isopeptides, only ET-1 is produced by endothelial cells.[47] The ability of this substance to produce sustained, powerful vasoconstriction is associated with the activation of smooth muscle membrane ET receptors leading to a phospholipase C (PLC)-dependent elevation of intracellular calcium.[21] Factors that stimulate the production of ET-1 include vasopressin, angiotensin II, thrombin and haemoglobin, concentrations of which are elevated in the cerebrospinal fluid (CSF) following SAH.[47] ET-1 may also be produced by vascular smooth muscle cells.[48] Although the precise role of ET-1 in posthaemorrhagic arterial narrowing is controversial,[47–49] Seifert et al[5] suggest that there is an elevated concentration of ET-1 in the CSF of patients with aneurysmal SAH, and that this correlates temporally with the development of vasospasm. Another study[48] has reported increased expression of ET receptor mRNA on day 3 of experimental SAH, paralleling the hypersensitivity to ET that occurs within 48–72 h of the ictus. On the other hand, Cosentino et al[49] have reported that intracisternal injection of either the ET receptor antagonist BQ-123 or the ET converting enzyme inhibitor phosphoramidon does *not* prevent SAH-induced vasospasm.

Oxygen derived free radicals

Following SAH, oxyhaemoglobin autoxidation leads to the production of oxygen derived free radicals, in particular superoxide.[50] Superoxide is a potent inactivator of NO.[50,51] In experiments using the canine basilar artery, Katusic et al[52] have shown that free radicals generated by xanthine oxidase mediate endothelium-dependent contractions by stimulating the activity of vasoconstrictive substances such as prostaglandin H_2 (PGH_2) and thromboxane A_2 (TXA_2), while inhibiting the basal production of vasodilators such as NO. These findings have been supported by other studies showing that the presence of L-NMMA augments contractions to free radicals,[52] whereas the opposite effect is achieved in the presence of L-arginine.[50] It should be noted that superoxide also suppresses the production of the vasodilator PGI_2.[51] In support of the aforementioned experimental work, Clarke-Haley et al[53] have found that in patients suffering from aneurysmal SAH, the use of the free radical scavenger, tirilazad, is associated with an improvement in overall 3-month outcome.

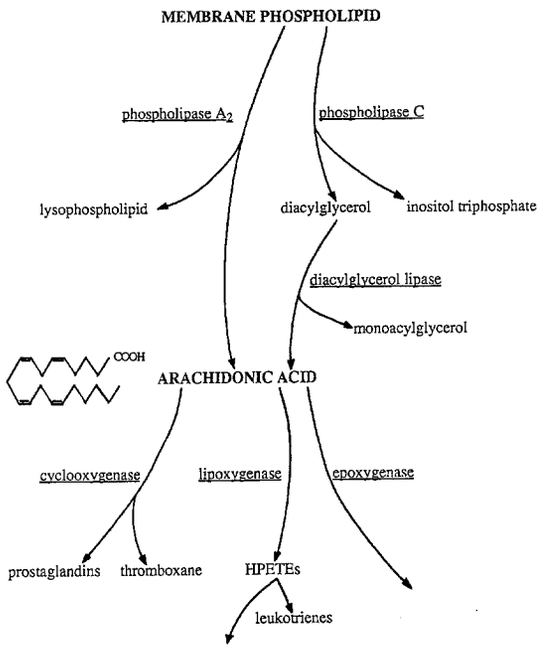

Fig. 2 Formation and catabolism of AA. AA is formed from membrane phospholipid by one of two enzyme mediated pathways (phospholipase A_2 or phospholipase C). Once formed, it is converted into substances such as prostaglandins, thromboxane and leukotrienes through the actions of the enzymes cyclooxygenase, lipoxygenase and epoxygenase. The vasoactive properties of numerous cyclooxygenase derivatives are well recognized.

Arachidonic acid

The formation and catabolism of AA, a membrane derived polyunsaturated fatty acid, is illustrated in Figure 2. The production of AA or one of its non-cyclooxygenase metabolites has been shown to be integrally related to the cytotoxicity of oxyhaemoglobin,[14] while the vasoactive properties of the cyclooxygenase derivatives of AA, especially PGI_2 and TXA_2, are also well recognized.[32,51] At a subcellular level AA has been shown to inhibit the activity of neuronal calcium channels.[54] Furthermore, evidence for a final common pathway between this substance and NO has been widely proposed,[55–58] although the precise point of interaction remains unknown.[55]

Vascular smooth muscle layer

Contractile apparatus

Relaxation of smooth muscle, like contraction, is an energy dependent process.[59,60] Hydrolysis of adenosine triphosphate (ATP) provides the energy through which the cross-bridge bonding between myosin and actin is reversed. Furthermore, the tonic state of the contractile apparatus is also dependent upon the levels of intracellular calcium[59,61] and magnesium.[20,59] While contraction involves rising intracellular calcium and the hydrolysis of ATP by calcium-dependent ATPase, relaxation is dependent upon falling intracellular calcium and ATP hydrolysis by magnesium-dependent ATPase.[59] It is therefore conceivable that following aneurysmal SAH, any alteration in the cellular availability of ATP, calcium or magnesium may contribute significantly to the development of CVSP.[6,59]

Inducible NOS

The features of the constitutive form of NOS have been described in detail above. As reviewed by Änggård,[22] the existence of an inducible isoenzyme, inducible nitric oxide synthase (iNOS), has also been recognized. Unlike cNOS, iNOS is calcium-*in*dependent and may be produced within vascular smooth muscle cells in certain disease states.[11,22] Enhanced production and release of inflammatory cell cytokines such as IL-1β occur in SAH and stimulate the de novo synthesis of iNOS in vascular smooth muscle cells.[11] Under these conditions, excessive NO production may contribute to cellular dysfunction or be overtly cytotoxic.[11,21,22]

Calcium channels

As in the case of neuronal calcium channels,[62] it is generally agreed that there are at least two different types of voltage gated calcium channels (VGCCs) present in vascular smooth muscle cells, one being the slowly-inactivating L-type calcium channel and the other the more rapidly inactivating N-type.[61,63] In both preparations, their modulation by substances such as NO and AA have been characterized.[54,55,61] In vascular smooth muscle, NO has been found to cause relaxation by reversibly inhibiting the influx of calcium through VGCCs while promoting its uptake into the sarcoplasmic reticulum.[61] AA also inhibits VGCCs, possibly via its lipoxygenase metabolites.[54] By contrast, ET-1 leads to muscle contraction via accelerating calcium influx through VGCCs, an effect which occurs in addition to its ability to increase calcium release from intracellular stores.[64] Finally, it should be noted that dihydropyridines such as nifedipine and nimodipine have been shown to modulate the activity of neurovascular VGCCs,[61,62,65] a finding that accounts for much of their clinical efficacy.[9,66]

Potassium channels

The opening of vascular smooth muscle potassium channels facilitates relaxation by hyperpolarising the cell membrane, thereby closing its voltage gated calcium channels.[6] The existence of an endogenous substance indirectly mediating vasodilation through the opening of vascular potassium channels has been proposed (for reviews see Shepherd et al[15] and Shepherd and Katusic[32]). In support of this hypothesis it has recently been shown that cerebral blood vessels possess ATP sensitive potassium channels whose activation by exogenous agents such as bimakalim and cromakalim is associated with vasodilation.[67]

Adventitial nerves

Adrenergic innervation

The presence of sympathetic nerve fibres innervating cerebral blood vessels has been well described.[68] Here,

the density of adrenergic nerve fibre innervation decreases proximodistally.[69] Stimulation of these fibres correlates strongly with vasoconstriction,[68,69] and this is achieved by either chemical or electrical coupling; that is, either via noradrenaline activation of smooth muscle α-adrenoceptors causing contraction through the release of calcium ions from intracellular stores, or via production of excitatory junction potentials which can summate to activate smooth muscle cell VGCCs. Although the former mechanism predominates under normal conditions, in pathological states associated with vascular smooth muscle cell proliferation, such as hypertension, there is a significant shift from chemical to electrical signal transduction.[69] Direct structural and functional damage to perivascular neurons following SAH has been widely documented.[7,14,59,70–72] Such damage corresponds closely to a reduction in the number of adrenergic fibres innervating cerebral blood vessels under such conditions.[7] Furthermore, the loss of adrenergic neural input has been coupled to increased levels of adrenergic receptor sites on cerebral vessels, the functional correlate being denervation hypersensitivity to circulating or locally released biogenic amines.[7]

Non-adrenergic, non-cholinergic nerve fibres

It is now widely recognized that cerebral blood vessels are endowed with populations of neuronal fibres releasing a wide variety of substances.[73] It has been shown that stimulation of certain non-adrenergic, non-cholinergic (NANC) neurons induces relaxation of arterial segments in vitro and an increase in cortical blood flow in vivo.[46,72,74,75] Immunohistochemical[34,74] studies have indicated that NO is the major neurotransmitter released from these perivascular 'nitrergic' neurons. Support for this finding has come from a number of physiological studies,[72,75,76] which show that transmural nerve stimulation (TMNS) of such NANC fibres induces relaxation of isolated cerebral arteries, an effect blocked in the presence of L-NMMA and L-NAME, but facilitated by the addition of L-arginine.[72,74] As with adrenergic fibres, posthaemorrhagic nitrergic neuronal damage also occurs,[70,72] and the cytotoxicity of haemoglobin has been strongly implicated in this process.[14] Further, haemosylate or haemoglobin is known to abolish the NANC fibre-mediated relaxation induced by TMNS in experiments using isolated cerebral arteries, while the presence of superoxide dismutase enhances it.[74]

Carbon monoxide

In 1993, Verma et al[37] reported a putative neuromodulatory role for carbon monoxide (CO). This substance, another short-lived and readily diffusible gas, is formed by the action of the enzyme 'heme oxygenase' and, like NO, activates GCase to regulate cGMP.[37,77–79] Further, through in situ hybridization, heme oxygenase (in particular the constitutive isoenzyme heme oxygenase-2) has been localised to discrete neuronal populations throughout the brain.[80] Here, its mRNA has been shown to be co-localized with that of GCase, suggesting that a substantial portion of GCase may in fact serve as a target for CO.[81] Like NO, CO has been shown to relax smooth muscle and inhibit platelet aggregation, and the presence of heme oxygenase within vascular smooth muscle cells has also been demonstrated.[82] Finally, cytochrome p-450 reductase serves as an electron donor to heme oxygenase.[36,37] The link between NOS and heme oxygenase is illustrated in Figure 3. Although it is yet to be established, certain perivascular nerves may secrete CO as a modulator of cerebrovascular tone and platelet activity.

Calcitonin gene-related peptide

Perivascular nerves also release calcitonin gene-related peptide (CGRP), a vasoactive substance that relaxes vascular smooth muscle.[63] The action of CGRP is associated with elevated levels of cAMP,[71] an effect which may decrease the sensitivity of the vascular smooth muscle cell to spasmogens.[83] CGRP also causes an inhibition of PKC-mediated vasoconstriction.[71,84,85] Experimental models of SAH have shown an initial fall in CGRP-like immunoreactivity followed by its reappearance within 4–8 weeks.[86,87] In support of this finding, a number of studies have shown an association between CVSP and elevated PKC activity,[88,89] with the time course of PKC activation closely correlating to that of cerebroarterial narrowing.[84,89]

Part 2 – Structural changes

Since the early autopsy series of Crompton in 1964,[90] much study has been given to the vasculopathy associated with cerebral vasospasm following aneurysmal SAH (for

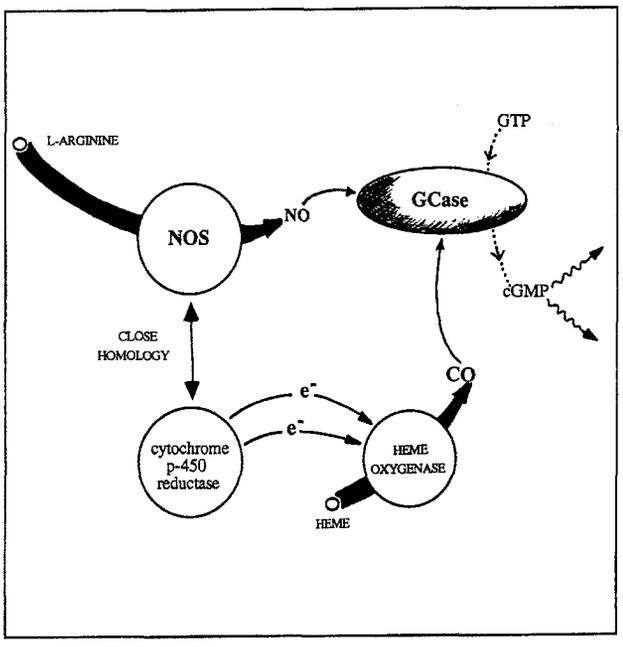

Fig. 3 The link between NOS and heme oxygenase. Cytochrome p-450 reductase, an enzyme that bears close homology to NOS, serves as a donor of electrons (e^-) to heme oxygenase. NOS forms NO while heme oxygenase forms CO; both of these enzymes activate the same enzyme (GCase) to elevate cGMP levels.

Table 3 Posthaemorrhagic pathological changes by compartment

Subarachnoid space	Vascular smooth muscle	Vascular endothelium	Adventitial nerves
Formation of thrombus	Myocontraction and myonecrosis	Cell distortion	Fibrosis
Clot lysis	De-differentiation	Internal elastic lamina convolution	Inflammatory cell infiltration
Microhaemorrhages	Myofibroblast proliferation	Invasion by myofibroblasts	Neuronal damage
		Subintimal collagen deposition	

reviews see Mayberg[7] and Weir[91]). As elaborated below, distinct pathological changes develop in each of the four cerebrovascular microcompartments. These are summarized in Table 3.

Subarachnoid space

Perhaps the most significant immediate structural alteration to the subarachnoid space (SAS) following aneurysmal rupture is the formation of blood clot within it.[1] This process, accelerated by factors present within the CSF,[92] has been widely regarded as being the triggering step of CVSP.[1,70,93] The thrombus is inherently vasoactive. Included within it and the unclotted blood permeating the SAS are numerous cellular and proteinaceous fractions of whole blood.[7] Of these, haemoglobin may be the most important.[1,7] Irreversible haemoglobin deposition within the arterial wall has been shown to occur in chronic SAH.[94] Further, it should be noted that within the first 3 weeks of the ictus, multiple small haemorrhages begin to occur into the SAS from the growth of tenuous capillaries into the newly formed clot.[92]

Vascular endothelium

Endothelial cell damage following SAH has been well documented.[1,14] In addition to platelet adhesion and the formation of microthrombus, light microscopic studies have shown that exposure of endothelial cells to the breakdown products of blood is associated with endothelial cell distortion, convolution of the internal elastic lamina, invasion by myointimal cells, and subintimal deposition of collagen and fibroblasts.[7,9,14,70] Furthermore, using electron microscopy, folding and vacuolation of the endothelium, changes in endothelial basement membrane structure, loss of interendothelial tight junctions, and thickening and corrugation of the intima have also been demonstrated.[7,9]

Vascular smooth muscle layer

The most well recognized posthaemorrhagic changes to the tunica media include contraction, vacuolation and degeneration of smooth muscle cells leading to myonecrosis.[9,14,70] In addition, the vascular myocyte undergoes phenotypic *de*differentiation, assuming certain features of a fibroblast,[7] with the ability to proliferate in response to mitogens such as PDGF.[95] Ultrastructural correlates of this dedifferentiation include the loss of contractile proteins and a prominent increase in type-1 collagen production.[96,97] It is of interest to note that in vasospastic cerebral arteries the cytotectural disruption of the tunica media caused by mechanical angioplasty[98] and pulsed dye laser therapy[99] may account for the finding that vessels treated by such procedures do not reconstrict.

Adventitial nerves

Following SAH, the adventitia undergoes fibrosis and inflammatory cell infiltration,[9] and there is a marked reduction in the number of perivascular nerve fibres containing vasoactive compounds such as NO,[70] catecholamines[7] and CGRP.[71] It is also of interest to note that the adventitia of cerebral arteries is more porous than that of other types.[100] This feature may not only facilitate the diffusion and vasoconstrictive effects of compounds such as haemoglobin but, in models of acute SAH, may also account for the reversibility of their actions via washout.[70]

Part 3 – A binding hypothesis

Despite exhaustive clinical and experimental research the precise biological mechanism underlying cerebrovascular spasm remains unknown although it is almost certainly multifactorial. Two general points, however, may be made: firstly, haemoglobin is the most likely triggering factor; and secondly, a link between neurovascular NO, AA and CO systems may greatly help to explain the basis of this condition.

Haemoglobin triggers vasospasm

Arterial narrowing begins approximately 3 days after the ictus, reaches a peak at 6–7 days (whence it may become clinically apparent)[3] and reverts by about day 14.[1] Temporally, this correlates well with clot lysis and the release of vasoactive agents, especially haemoglobin.[1,70] Once formed, haemoglobin is intensely vasculopathic, its far reaching effects facilitated by a porous adventitia.[100] At a structural level this substance is directly cytotoxic to the endothelial and smooth muscle layers of the vessel and is damaging to perivascular nerve fibres.[7,9,14,70] The functional correlates of its cytotoxicity are secondarily impaired endothelium- and nerve-mediated vasodilation[1,12] and directly facilitated myocontraction, myonecrosis and medial fibrosis.[1,7] In addition, haemoglobin enhances the formation of AA,[14] superoxide,[50] ET-1[47] and IL-1β,[11] lowers smooth muscle ATP levels,[60] and directly binds and inactivates NO and GCase.[43] As detailed above, each of these actions directly or indirectly contributes to vasoconstriction. Finally, the multiple microhaemorrhages that begin to occur into the SAS within a few weeks of the ictus may participate in maintaining the vasculopathy associated with chronic haemoglobin exposure.[92]

NO, AA and CO systems are linked

The widespread evidence for involvement of the NO, AA and CO systems in regulating basal arterial tone can be summarized as follows:

1. loss of NO action leads to impaired vascular relaxation while facilitating platelet aggregation and smooth muscle proliferation[16,30–32]
2. alteration in the balance between vasoactive AA metabolites such as PGI_2 and TXA_2 contributes to altered vascular tone, in addition to AA augmenting vascular smooth muscle contractions to noradrenaline[51] and being directly toxic to endothelial cells[32,51,52]
3. CO, like NO and PGI_2, relaxes blood vessels and inhibits platelet aggregation.[37]

That the NO, AA and CO systems are linked by some final common pathway is suggested by the findings of a number of studies. Gray et al[57] have indicated the ability of prostaglandin metabolites of AA to activate NOS, while an interaction between AA and NO at the level of GCase has been proposed by Gerzer et al[56] who found that AA, like NO, can increase cGMP levels. In support of this, Khurana and Bennett[54] have shown that the inhibitory effects of NO on neuronal calcium channels are similar to, and *non-additive* with those of AA. Recent work by Bredt et al[34,36] and Verma et al[37] demonstrates the link between NO and CO via the structural similarities of NOS and cytochrome p-450 reductase, the latter being biochemically coupled to heme oxygenase. CO, like NO and certain AA metabolites, also modulates cGMP levels via a direct action on GCase.[37] Further, cGMP and cAMP, which mediate the actions of NO, CO and AA, are linked through their co-regulation by intracellular calcium[59] and the ability of cGMP to modulate the activity of cAMP phosphodiesterase.[101] Finally, circumstantial evidence for a shared pathway between these systems is suggested by their strikingly similar effects: NO, CO and certain AA metabolites are all highly lipophilic compounds that cause vasodilation and inhibit platelet aggregation.

In conclusion, cerebrovascular spasm following aneurysmal SAH is a condition associated with significant morbidity and mortality. Although its precise pathophysiology remains elusive, two key components of its multifactorial nature are the deleterious presence of haemoglobin and a defect in the linked NO, AA and CO systems. These systems all converge at the level of the second messengers cGMP and cAMP. Elucidating the nature of their final common pathway beyond this point of convergence may significantly contribute to an improved understanding and more effective management of this life threatening condition.

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